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BACILLUS OF THE COLON-TYPHOID GROUP ISOLATED FROM A CASE OF FURUNCULOSIS

WADE W. OLIVER AND ALMA F. SCHWAB

*From the Department of Bacteriology, Hoagland Laboratory, Long Island College Hospital,
Brooklyn, N. Y.*

A colon-like organism has been isolated in pure culture from a case of furunculosis. The patient, who gave a history of annual or semi-annual crops of "boils," which had developed over a period of several years, presented, at the time of examination, three small acutely inflamed furuncles on the back of his neck. The largest lesion, which was red, indurated, and about the size of a dime, was punctured with a sterile needle, after preliminary cleansing of the skin with soap and water, mercuric chlorid (1:1,000) and alcohol. A small amount of a moderately thick, gray-white pus was expressed. Aerobic and anaerobic cultures were made on human blood agar (+1) and Martin's ascitic agar (0.5), and incubated at 37 C. After 24 hours' incubation, single colonies from the aerobic blood agar were streaked across plates of human blood agar (+1) and incubated aerobically at 37 C. At the end of 24 hours single colonies were plated in plain agar (+1), and after 24 hours more at 37 C., single colonies were transplanted to slants of plain agar (+1).

Morphology.—The organism was a gram-negative, nonmotile, noncapsulated, nonsporogenous rod, averaging about 2-3 mikrons in length and of about the thickness of the colon bacillus. Like the latter, it exhibited rather marked variations in size when grown for from 24 to 36 hours in plain infusion broth (+1). The majority of the forms were short single rods, with rounded ends. However, thin rods and small coccoid bodies were not infrequently present. The latter were more numerous in cultures incubated for a number of days (7-10). Certain organisms, especially the medium sized rods, showed bipolar staining and others exhibited two or three irregularly placed granules within the body of the rod.

Biology.—Growth occurred readily on ordinary mediums. On plain infusion agar (+1), after 24 hours' aerobic incubation at 37 C. the colonies were medium sized, round, with regular edges, grayish-white in color and slightly viscous. A moderately marked growth occurred in gelatin, with no liquefaction, the gelatin showing a moderate diffuse clouding with a well defined thin line of growth along the line of inoculation, after seven days at room temperature. Plain infusion broth (+1) showed a diffuse uniform clouding, after 24 hours at 37 C. No pellicle formed even after two weeks' continuous incubation.

After 2 days' incubation, a rather heavy gray-white collar of growth was visible on the sides of the test-tube immediately above the upper level of the broth, and in the bottom of the tube was present a moderate gray flocculent growth. No difference in the luxuriance of growth was noted in cultures incubated aerobically and at partial oxygen tension with the aid of *B. subtilis*. The organism was a facultative anaerobe, but even on human blood agar slants (+1), made anaerobic by the addition of pyrogalllic acid and potassium hydroxid, the growth was delicate. The optimum growth temperature was 37 C.

Products of Growth.—When grown aerobically on 1% sugar litmus-agar slants (+1) acid, but no gas, was split off from lactose, galactose, maltose mannite and saccharose, but glucose and dextrin remained unchanged even after two weeks' incubation. This test was made on +1 beef extract agar, previously rendered sugar-free by incubation with *B. coli*. The sugars, etc., after being sterilized for 15 minutes at 20 pounds' pressure, were added aseptically to the medium just before it solidified. Inoculations were made on the surface of the slant, as well as by a stab into the depths of the medium. At partial oxygen tension,¹ glucose-agar slants showed a distinct acid reaction, but no gas, after 36 hours' incubation at 37 C., as did the lactose, galactose, maltose, mannite, and saccharose. Dextrin was not fermented. Aerobic transplants made from the respective sugars to a fresh series of sugar agars which were incubated aerobically, gave the same readings as on the first series. The same thing held true in the case of the partial tension series.

After being transplanted every week to glucose (+1) and plain (+1) agar slants for six months, the organism was planted on a plain (+1) agar slant. After 24 hours' incubation at partial tension at 37 C. the tube was placed in the icebox, with the tube of *B. subtilis* attached, and left continuously in the refrigerator for 17 months. At the end of this time, a transplant was made to plain (+1) broth and a heavy growth of the bacillus was obtained after 24 hours' incubation. After determining by plating that the culture was pure, the organism was planted into a series of peptone water tubes to which had been added the respective sugars to a concentration of 1%. The Andrade indicator was employed, and to detect gas small inverted tubes within the broth were employed. In this series, after 24 hours' incubation at 37 C., a marked acid reaction, which persisted after at least 10 days' continuous incubation, but no gas, was observed in glucose, levulose, galactose, salicin, raffinose, saccharose, maltose, lactose, mannite, and glycerin (5%). A faint trace of acid, but no gas, was observed in dextrin after 24 hours' incubation, but after 5 days' continuous incubation at 37 C., the faint reddish tinge had completely disappeared from this tube except for the broth within the small inverted tube. One per cent. potato starch remained unchanged until the fifth day of continuous incubation at 37 C., at which time a faint reddish layer, indicating acid, was observed at the bottom of the tube. No gas was formed from the starch. On 6 days' incubation the starch showed a moderate acid reaction throughout the tube. Inulin remained unchanged. Litmus milk exhibited acid coagulation after 48 hours' incubation at 37 C. No indol was produced. Gelatin was not liquefied.

A macroscopic agglutination test with known typhoid antiserum gave a positive agglutination in a 1:20 dilution, but no agglutination in dilutions of 1:40, 1:80, and 1:160.

¹ Wherry and Oliver: Jour. Infect. Dis., 1916, 19, p. 288.

Pathogenesis for the Guinea-Pig.—After continuous cultivation for 5 months 0.5 c c of a 24-hour plain infusion broth (+1) culture was injected intraperitoneally into about a 300 gm. guinea-pig. Death resulted within 15 hours, the animal giving evidence of a profound toxemia. From the peritoneal fluid, which was scant, straw-colored and viscous, as well as from the heart's blood, the organism was recovered in pure culture.

Vaccine Therapy.—A vaccine was prepared from plain infusion agar (+1) four days after the isolation of the organism. The cultures were incubated at partial oxygen tension for 24 hours at 37 C. The vaccine was killed by heating in the water bath at 55 C. for one hour. The bacterial count was approximately one-half billion per c c of vaccine.

The reaction obtained on administration of the vaccine was strikingly severe. The initial injection consisted of 1 minim injected subcutaneously into the forearm. Within 4 hours a marked, tender, reddened, swollen, area developed at the site of the inoculation. The local reaction increased in intensity, so that within 24 hours a painful, very red, indurated area of about the size of a half dollar had developed. With the local reaction occurred a rather marked constitutional reaction. By the end of 24 hours, the temperature had risen to 101.5 F. and the following day the patient was incapacitated from work, complaining of headache, malaise, and venturing the assertion "I feel exactly the way I do when I am getting a new crop of boils."

The second injection, given after three days, consisted of 1 minim, and evoked a slightly less marked local reaction. Then the dosage was increased 1 minim per injection at three day intervals, until 14 minims were being given at an injection. Injections of this maximum dosage were continued every three days until about 4 c c had been administered. After the first three injections the reaction was confined to a moderate local one.

The vaccine therapy seemed to abort the initial lesions, and no lesions have developed up to the present. It is now twenty months since the initial injection.

DISCUSSION

Pollitzer² says: "A boil is produced in one way and in one way only. It is the result of an infection of the skin with the staphylococcus aureus by way of the hair follicles." McDonald³ reports twenty-eight cases of furunculosis, in all of which the staphylococcus aureus or albus was found. Similar findings were reported by Compton⁴ and Harris.⁵

From its morphology and from certain of its cultural characters, the organism seems to belong within the colon group of bacteria. The finding of the bacillus in pure culture from the lesions of the disease and the marked reaction set up in the patient when treated with an

² The Post Graduate, 1914, 29, p. 825.

³ Arch. Pediat., 1911, 28, p. 772.

⁴ Lancet, 1918, 1, p. 99.

⁵ N. Y. Med. Jour., 1911, 94, p. 984.

autogenous vaccine suggest an etiologic relation of the organism to the disease. The marked toxicity of the bacillus for a guinea-pig is also worthy of note.

The action of the organism on glucose merits notice. The fermentation of glucose at partial oxygen tension and its nonfermentation under aerobic conditions bears a resemblance to the fermentation reactions on this sugar of a bacillus isolated by one of us from the feces of a case of dysentery.⁶ The production of acid in glucose broth is explainable possibly by the fact that in the depth of the broth the bacillus found conditions of diminished tension which favored splitting of the sugar.

SUMMARY

A member of the colon group of bacteria was isolated in pure culture from a case of furunculosis.

Intraperitoneal injection of 0.5 c c of a 24-hour broth culture, after the organism had been cultivated continuously for 5 months, resulted in the death of a guinea-pig of about 300 gm. in weight, in 15 hours.

Under aerobic conditions on solid media the organism failed to produce acid from glucose, even on prolonged incubation (14 days), but under conditions of partial oxygen tension, glucose was fermented with acid production, but no gas, within 36 hours. Under both aerobic and partial tension conditions, acid, but no gas, was formed from lactose, galactose, maltose, mannite, and saccharose.

The marked local and general reaction on the part of the patient following the initial injection of 1 minim of an autogenous vaccine (about one half billion bacilli per c c) followed by the patient's freedom from boils for almost two years since the vaccine was administered, suggest a relationship of the bacillus to the disease.

⁶ Oliver and Perkins: *Jour. Infect. Dis.*, 1918, 22, p. 507.